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THE KINETICS OF SALIVARY ELIMINATION OF CYCLOPHOSPHAMIDE IN MAN

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1 The concentrations of cyclophosphamide in plasma and saliva were determined in seven patients following administration of single doses of cyclophosphamide during chemotherapy for lymphoma.

2 The saliva/plasma ratio was 0.77 ± 0.24 (s.d.) and showed no time-dependence being rapidly established following intravenous and oral administration.

3 The $T_{\frac{1}{2}}$ of cyclophosphamide (8.38 ± 2.25 h) determined from salivary measurements was not significantly different from that in plasma (8.21 ± 2.60 h). It was not possible to estimate the apparent volume of distribution or total body clearance utilizing the salivary cyclophosphamide concentration without appropriate correction for the saliva/plasma concentration ratio.

4 The binding to the plasma protein of normal plasma of cyclophosphamide was $13.4 \pm 5.3\%$. The Scatchard plot for binding to bovine serum albumin indicates only weak binding to non-specific sites.

5 Salivary cyclophosphamide therefore indicates the concentration of the unbound fraction of plasma cyclophosphamide.

Introduction

The presence of drugs in saliva subsequent to administration of a normal therapeutic dose has been demonstrated for a number of compounds in clinical use (Horning, Brown, Nowlin, Lertratanangkoon, Kellaway & Zion, 1977; Danhof & Breimer, 1978). Studies of drug pharmacokinetics may sometimes be usefully made using saliva rather than plasma samples. Although cyclophosphamide is one of the most widely used antineoplastic agents, its pharmacokinetics have not been commensurately investigated. Cyclophosphamide has previously been identified using mass-spectrometry in samples of human saliva by Duncan, Colvin & Fenselau (1973). It was therefore thought worthwhile to determine whether saliva could be used instead of plasma sampling to follow the kinetics of cyclophosphamide and thereby reveal the variability in drug disposition which is known to occur between patients (Bagley, Bostick, & De Vita, 1973; Juma, Rogers & Trounce, 1979).

Methods

Cyclophosphamide was administered in a single intravenous or oral dose to seven patients undergoing chemotherapy for stage III–IV lymphoma. All patients received vincristine and prednisolone concurrently as part of their treatment protocol. A total of 15 studies was made. Simultaneous samples of venous blood and unstimulated mixed saliva were collected at appropriate time intervals following dosing. Plasma was separated from the blood and this and the saliva samples were stored at −20°C until analysed for unchanged cyclophosphamide. Preliminary experiments indicated that cyclophosphamide concentrations are unchanged in these fluids for at least 2 months under such conditions. Plasma and salivary cyclophosphamide concentrations were estimated by synthesis of the trifluoracetyl derivative and subjecting this to gas-chromatography using alkali flame ionization detection according to the procedure of Juma, Rogers, Trounce & Bradbrook (1978).

Cyclophosphamide binding to plasma protein was investigated using plasma from normal drug-free subjects which was incubated at 37°C for 1 h with cyclophosphamide to give final concentrations between 7 and 35 μg/ml. These plasma solutions were then transferred to Amicon Diafo® ultrafiltration cones (Amicon Ltd, Bucks). Preliminary experiments having shown that cyclophosphamide is not bound to these membranes, ultrafiltration was then carried out by gentle centrifugation at 500 *g* at 37°C until less than 5% of the fluid contained in the cones had been filtered. Cyclophosphamide levels were then estimated in the filtrate and the plasma by gas

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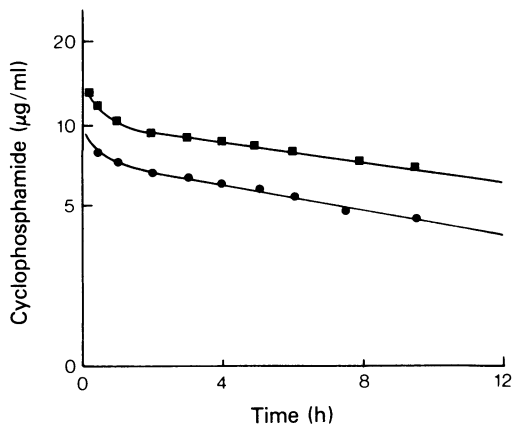

Figure 1 Plasma (■) and salivary(●) cyclophosphamide concentrations as a function of time after intravenous administration of 800 mg cyclophosphamide to a patient weighing 86 kg.

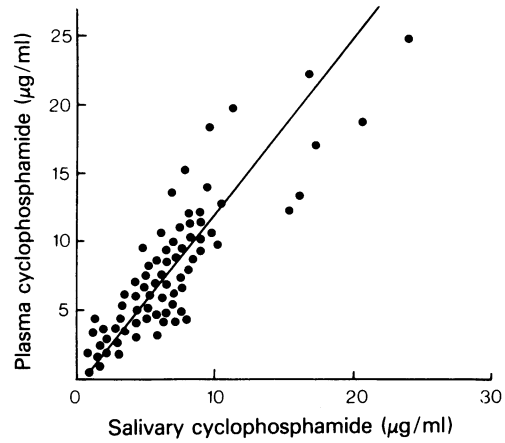

Figure 2 Correlation between the plasma and saliva concentrations of cyclophosphamide in seven patients over all fifteen studies ($n = 86$).

chromatography. A Scatchard plot (Scatchard, 1949) for binding of cyclophosphamide to albumin was constructed by the same procedure using purified bovine serum albumin (Sigma Ltd, Poole, Dorset) at a concentration of 0.4% (kept low to prevent dimerization) in 0.067 M phosphate buffer (pH 7.4).

Plasma and salivary cyclophosphamide kinetics were found to be best fitted by an open two compartment pharmacokinetic model for intravenous doses and an open one compartment model in the case of oral administration. The data were fitted to the appropriate equations as detailed by Juma *et al.* (1979) using the simplex non-linear least squares digital computer program of Nelder & Mead (1965).

Statistical comparisons were made by Student's *t*-test.

Results

Good correlation between plasma and salivary cyclophosphamide concentrations was observed in each patient studied. As an example, Figure 1 shows the cyclophosphamide concentrations in plasma and saliva of an individual patient following an intravenous dose of cyclophosphamide. There was no significant difference between plasma and salivary half lives (Table 1). The apparent volumes of distribution and total body clearances were also calculated from plasma and saliva estimations using the appropriate pharmacokinetic models. The salivary estimates of these parameters were significantly different from those based upon plasma determinations (Table 1). The mean ratio of the area under the salivary concentration, time curve and the area under the plasma concentration, time curve was 0.76 ± 0.31 (s.d.) $\mu g\, ml^{-1}\, h$. Correction of the salivary levels to their corresponding plasma values by this factor resulted in estimates of the pharmacokinetic parameters which were not significantly different from those determined by plasma estimations.

Corresponding plasma and salivary cyclophosphamide concentrations for all seven subjects are

Table 1 Pharmacokinetic parameters derived from plasma and salivary cyclophosphamide estimations

	Plasma		*Saliva*		
	mean	*s.d.*	*mean*	*s.d*	
Elimination half-life (h)	8.21	2.60	8.38	2.25	NS
Disposition rate constant β (h^{-1})	0.091	0.026	0.100	0.038	NS
Elimination rate constant k_{10}, for intravenous administration only (h^{-1})	0.167	0.080	0.275	0.301	NS
Apparent volume of distribution ($l\, kg^{-1}$)	0.70	0.086	0.94	0.198	$P<0.05$
Total body clearance ($l\, h^{-1}\, kg^{-1}$)	3.97	0.64	5.80	2.23	$P<0.05$

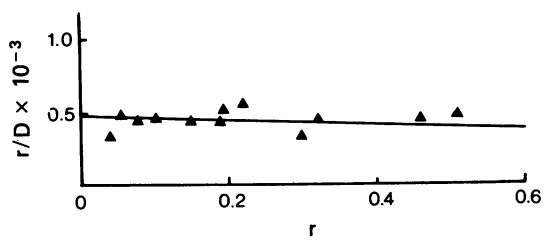

Figure 3 Scatchard plot of studies on cyclophosphamide binding to bovine serum albumin *in vitro* at 37°C. The abscissa r is the molar ratio of cyclophosphamide bound to albumin. The abscissa r/D is r divided by the molar concentration of free cyclophosphamide D.

shown in Figure 2. The overall correlation coefficient was 0.89 ($P<0.001$) and the intercept was not significantly different from zero.

The mean saliva/plasma concentration was 0.77 ± 0.24 (s.d.). This was not significantly different from the ratio of the corresponding areas under the concentration, time curves. The relationship between the plasma and saliva concentrations was explored further by plotting these variables in a time-sequence according to the procedure of Galeazzi, Benet & Sheiner (1976). The area included in the resultant 'hysteresis-loop' was not significantly different from zero indicating that equilibrium between plasma and saliva was quickly established.

Plasma protein binding was found to be $13.4 \pm 5.3\%$ (s.d. for six observations). The Scatchard plot for binding to bovine serum albumin is shown as Figure 3.

Discussion

A linear relationship has been demonstrated over a wide concentration range between the plasma and salivary concentrations of cyclophosphamide. This relationship may be exploited for pharmacokinetic purposes since the concentration of cyclophosphamide in saliva is proportional to that in plasma. The ratio of cyclophosphamide concentrations between plasma and saliva is rapidly established and shows no time dependency. This is in contrast to the situation which obtains for procainamide (Galeazzi *et al.*, 1976), theophylline (Koysooko, Ellis & Levy, 1974) and caffeine (Newton, Lind, Morrison, Rogers & Bradbrook, unpublished observations) but is consistent with our previous pharmacokinetic studies of cyclophosphamide which indicate a relatively rapid transfer of drug between peripheral and central compartments (Juma *et al.*, 1979).

Unfortunately the observed saliva/plasma ratio does not appear to be close enough to unity to allow realistic estimates of apparent volume of distribution or total body clearance to be made without a correction for the saliva/plasma concentration ratio. If this correction is made, and it is possible to do this on the basis of a single pair of plasma and saliva samples, the salivary kinetics will accurately predict the plasma kinetics.

Salivary drug concentrations are indicative of the free drug concentration in plasma (Horning *et al.*, 1977; Danhof & Breimer, 1978) and this would appear to apply to cyclophosphamide. Our estimate of the free fraction of cyclophosphamide in normal plasma is about 87%. This is in approximate agreement with the salivary/plasma concentration ratio found in the patients studied. Bagley *et al.* (1973) found that 12–14% of radio-labelled cyclophosphamide was bound to plasma protein in samples from their patients. Jardine, Fenselau, Appler, Kan, Brundrett & Colvin (1978) however, found 24% cyclophosphamide binding in a single patient study. This was carried out at 4°C with frozen plasma samples which may explain the discrepancy of their findings. The Scatchard plot for binding of cyclophosphamide to bovine serum albumin indicates only weak binding to non-specific sites on the albumin molecule since the line of best-fit to the experimental points is almost parallel to the abscissa. Since cyclophosphamide is an unionized molecule it is unlikely that the pH of saliva will substantially alter the amount of drug eliminated by this route.

Cyclophosphamide is not in itself an alkylating agent and requires activation to various active metabolites to become effective. Studies of its plasma level therefore give an indirect estimate of its therapeutic efficacy. The present work suggests that salivary cyclophosphamide estimations are suitable for establishing the disposition and elimination rate constants, which mainly reflect biotransformation. They are unsatisfactory, however, for the determination of the complete disposition kinetics of the drug unless appropriate corrections are made.

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